

MALDI matrix cluster ions as internal references for ion mobility measurements

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Abstract:

In the absence of high vacuum, the mobility of ions in an electric field is dependent on the charge, mass, shape and size of the ions. To achieve accurate calibration for ion mobility measurements, it is important to select calibrants with comparable physical properties to the molecular ions of interest. The size of an ion is often referred as its collision cross section (CCS). Currently, the information on the CCS values of various types of molecular ions are limited, thus representing a challenge to the calibration of ion mobility spectrometry. In this study, instead of finding a way to improve the calibration of ion mobility measurements, the intrinsic by-products of the conventional matrix-assisted laser desorption/ionization (MALDI) technique, namely MALDI matrix cluster (MAC) ions, are being used as internal references for ion mobility measurements. The standard MALDI matrix and sample preparation method are used. During the MALDI ionization process, MAC ions are generated and co-exist with the molecular ions of interest within the ion source. Our results indicate the MAC ions do possess suitable ion mobility characteristics, thus allowing the MAC ions to serve as internal reference for ion mobility measurements. The MAC ions cannot be used as internal standard or calibrants for ion mobility measurements, because their molecular structures as well as their CCS are unknown. However, the detection of MAC ions can allow us to determine whether the normal operation as well as the expected performance on the sensitivity and resolution of ion mobility spectrometry are achievable and reproducible. The MAC ions can also facilitate the transfer of specific experimental protocols between ion mobility instruments and/or laboratories. No extra materials, equipment or procedure are required for using MAC ions. For the proof of concept, all experimental work in this study was carried out on a traveling wave ion mobility mass spectrometry platform.

Keywords: Ion mobility | MALDI | Matrix | Internal reference

Article:

Introduction

The analytical measurement of ion mobility was first reported by Tyndall in 1928 [1]. With the development and commercialization of ion mobility spectrometry, there have been a continuous growth on using the technique to analyze different types of molecules [2]. In general, an ion mobility spectrometer can be divided into three major components. The first component is an ion source where samples are introduced and ionized. The ions are then transmitted to an ion mobility cell where different ions are physically separated. To complete the analysis, the ions are detected by using an ion detector. Through the advances on exerting an electric field within the ion mobility cell, there are different ways to control the mobility of ions. These had led to the development of different ion mobility technologies, some of which have been commercialized that include drift tube, traveling wave, differential ion mobility and trapped ion [2,3,4]. For a complete list of the current ion mobility technologies, readers are referred to a review written by Figueras and his associates [5].

The detection of ions upon their separation in ion mobility spectrometry provides an opportunity for us to measure the accurate mass of ions. The coupling of ion mobility spectrometry to mass spectrometry can provide extra capacity to resolve more complex mixtures, especially those contain isomeric compounds. Another benefit from the coupling of ion mobility spectrometry to mass spectrometry is the access to a variety of ionization techniques that were originally developed for mass spectrometry. It is important to note that the intrinsic chemical and/or physical properties of chemical compounds can be vastly different from each other. Hence, in order to ensure sufficient level of ion formation can be achieved when different types of sample are being analyzed, the possibility of using a different ionization technique can be beneficial. Currently, the most versatile platform for ion mobility mass spectrometry in terms of the variety of ion source is the Synapt series manufactured by Waters [6]. Matrix-assisted laser desorption/ionization (MALDI) is one of the switchable ion sources available on the Synapt platform.

The invention of MALDI and its applications have played a major role in the transition from the classical destructive mass spectrometry to non-destructive mass spectrometry, which has paved way to the successful introduction of tandem mass spectrometry. For MALDI mass spectrometric measurements, the use of MALDI matrix is essential. An excess amount of MALDI matrix is mixed with a sample of interest, and the mixture is crystallized on a sample plate. During the MALDI process, the sample-matrix co-crystals are irradiated by UV laser pulses. The MALDI matrix can effectively absorb the high laser energy, thus shielding the compounds of interest from the destructive laser irradiation. Following the UV absorption, the temperature of MALDI matrix reaches its boiling point, and some of the matrix molecules are dissociated into fragments. The gas-phase recombination of those fragments leads to the formation of a series of matrix cluster (MAC) ions that have different mass-to-charge ratios [7]. Conventionally, the MAC ions are considered as interference to the ions of interest and may lead to signal suppression in MALDI mass spectrometric measurements. On the other hand, MAC ions can support the ionization of some chemical compounds by proton transfer [8]. Also, the presence of matrix cluster ions can help to cool down the ions of interest within the ion source [9].

In order to achieve accurate analytical measurements, proper calibration of the instrumentation must be carried out. This applies to all ion mobility technologies including the drift tube technology despite the fact that measured drift time of a specific ion in a drift tube can be directly converted into its collision cross section. This is because the mobility of a specific ion is dependent on the gas pressure and temperature inside the drift tube [2]. In practice, it is not always easy to maintain both parameters at constant levels and/or trust the gauge readouts of both parameters (if available) have been properly calibrated. Also, the purity of the most commonly used nitrogen buffer gas for ion mobility measurements may vary in different laboratories, which can lead to the variation of drift time measurements [10]. The mobility of ions in the gaseous phase in absence of high vacuum is also dependent on the charge, mass, and size of ions [2]. It is, therefore, important to match all three parameters between the compound of interest and the calibrants. The conformation or shape of ions would also affect the mobility of ions. In this study, instead of searching for the most suitable ion mobility calibrants or develop a new strategy for performing calibration [11,12,13,14,15], we are exploring a novel approach that will allow us to determine whether sufficient reproducibility in the ion mobility measurements is achieved which, in turn, will allow us to attain higher confidence on the identification of specific ions. The use of existing MALDI matrix cluster ions as internal references for ion mobility measurements is thus explored. For the proof of concept, all experimental work in this study was carried out on a traveling wave ion mobility mass spectrometry platform.

Materials and methods

All MALDI matrices, ammonium citrate dibasic sodium iodide, HPLC-grade acetonitrile (ACN), trifluoroacetic acid (TFA), and Bioultra polyethylene glycol 1000 (PEG) were purchased from Sigma Aldrich (St. Louis, MO). A small peptide ($\geq 95\%$ purity) was ordered from AnaSpec Inc. (Fremont, CA). The sequence of the peptide was YSTCDFIM, which was cyclized between the sulfur group of cysteine residue and the C-terminal and has a monoisotopic mass of 960.3721 Da. Water used in this study were double deionized with $\sim 18.5\text{ m}\Omega$.

Preparation of solutions

For α -cyano-4-cinnamic acid (CHCA) matrix solution, 20.0 mg of CHCA was dissolved in 1.0 mL of 50% ACN with 0.1% TFA. For 2,5-dihydroxybenzoic acid (DHB) matrix solution, 30.0 mg of DHB was dissolved in 1.0 mL of 30% ACN with 0.1% TFA [16]. For sinapinic acid (SA) matrix solution, 20.0 mg of SA was dissolved in 1.0 mL of 50% ACN with 0.1% TFA. For 3-hydroxypicolinic acid (3-HPA) matrix solution, 35.0 mg of 3-HPA and 8.80 mg of ammonium citrate dibasic were dissolved in 1.0 mL of 10% ACN with 0.1% TFA [17]. To dissolve the powder of each matrix compound, the matrix solution was vortexed 1-2 min. Before use, all matrix solutions were filtered with 0.22 μm non-sterile Durapore (PDF) syringe membrane filter. Matrix solutions were kept away from direct sunlight and stored at $-20\text{ }^{\circ}\text{C}$ for no longer than 1 month. The PEG solution was prepared by mixing 10 μL of PEG with 30 μL of 2 g/L NaI and 190 μL of 50% ACN. The stock solution of peptide (100 μM) was prepared in 50% ACN, which was freshly diluted to 1 μM with 50% ACN and 0.1% TFA.

Ion mobility mass spectrometric (IM-MS) measurements

To prepare a MALDI sample, specific MALDI matrix solution was mixed with an equal volume of sample. 0.2 μ L of the mixture was then added on the surface of a clean stainless steel MALDI sample plate, and air-dried. For the measurements of MALDI matrix alone, 0.2 μ L of undiluted matrix solution was added on the sample plate and air-dried.

All experimental data were acquired by using a Waters Synapt G2 high definition mass spectrometer (Waters, Milford, MA) that was equipped with a switchable MALDI ion source. The instrument was calibrated by using sodium formate solution as recommended by the manufacturer. The instrument was operated under the MALDI mobility TOF MS mode for measuring either positive or negative ions. For the MALDI ion source, laser energy was set at 325 arb. Units, and laser firing rate was at 1000 per sec. The position of laser irradiation was manually adjusted until hot spots were found. The MALDI extraction and its adjacent hexapole bias were both set at 10 V. A mass range of 150 to 1200 Da was scanned in every second. Unless otherwise stated, the traveling wave ion mobility cell was operated under the default settings, which included a flow of Argon gas to the trap and transfer cell at 2 mL/min, a flow of Helium gas to the Helium cell at 180 mL/min, a flow of Nitrogen gas to the ion mobility cell at 90 mL/min, wave height at 40 V, and wave velocity at 650 m/s. The IMS bias voltage was set at 3 V. The TOF mass analyzer was operated under the resolution mode. In each experiment, the signals were integrated over 1 min of run time. With the switchable ion source, the pressure in the MALDI ion source was maintained at $\sim 10^{-5}$ Torr while the pressure in the TOF region was maintained at $\sim 10^{-7}$ Torr.

Results and discussion

Detection and characterization of matrix cluster (MAC) ions

Prior to using MAC ions as internal references for ion mobility measurements, the detection of MAC ions and its reproducibility were first evaluated. Without using any sample, CHCA matrix that has been commonly used for peptide measurements was measured. As shown in Fig. 1a, multiple peaks that correspond to the MAC ions of CHCA were easily detected in the mass spectrum. Although the molecular structure of each detectable MAC ion of CHCA is not known, the same mass spectral pattern in Fig. 1a, which includes both the presence of peaks at specific mass-to-charge (m/z) ratios and their relative peak intensities, was reproducible while there were sufficient matrix crystals and the laser intensity was maintained at the same level. When the m/z data in the mass spectrum of CHCA are combined with their corresponding drift time data, which is the time it took the ions to migrate through the ion mobility cell, a driftscope of CHCA can be plotted as shown in Fig. 1b. If a linear scale is used for the signal intensity in the driftscope, limited number of bands are observed (data not shown). By choosing a log scale for the signal intensity in the driftscope as shown in Fig. 1b, more discrete bands are observed. Although the low background noise is also picked up in the log scale, we find the presence of an overall trend line diagonally across the driftscope is easier to follow. Although some of the bands in Fig. 1b are not fully resolved, it does not prohibit the use of MAC ions as internal references (see later).

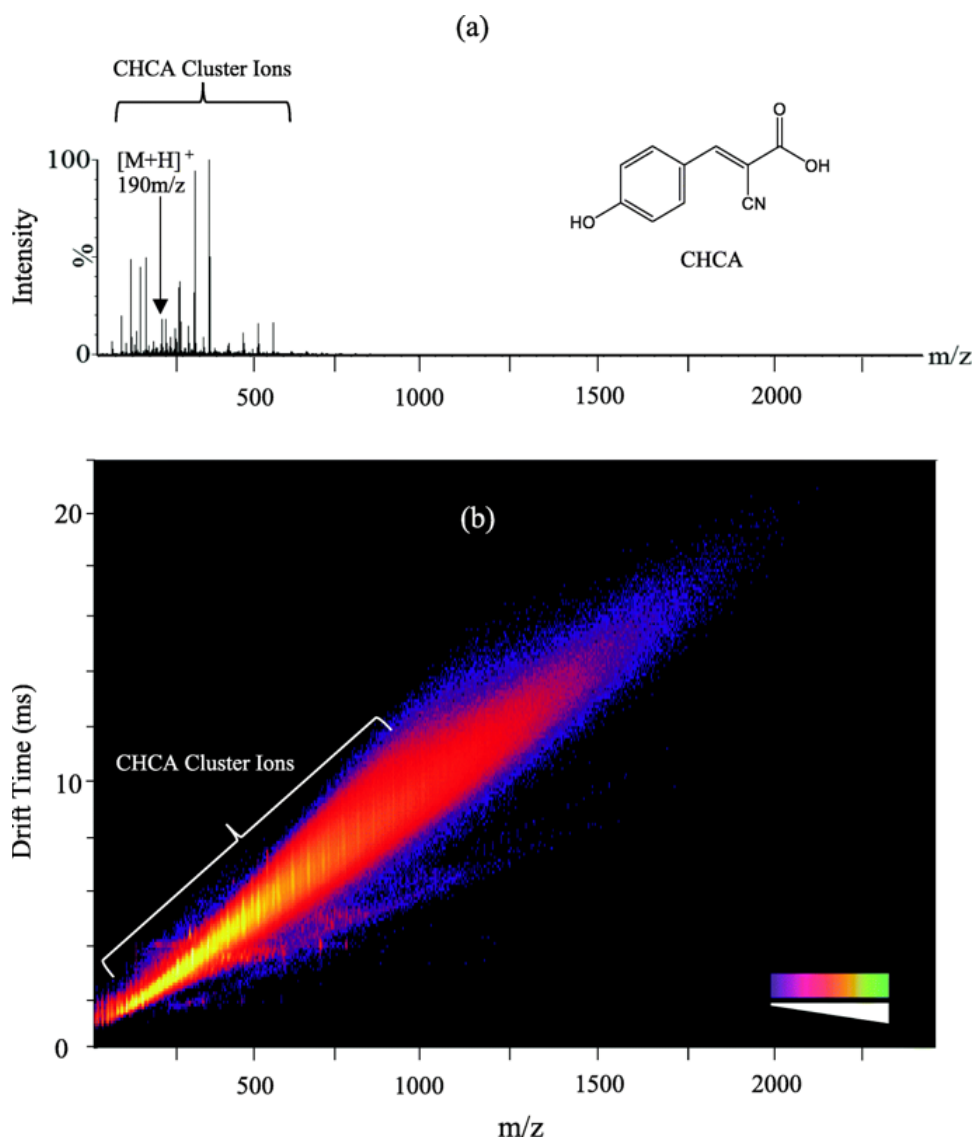


Figure 1. (a) Mass spectrum and (b) driftscope of α -cyano-4-cinnamic acid (CHCA) MALDI matrix ($[M + H]^+ = 190$ m/z) and its cluster ions. Positive ions were detected

Through the development of MALDI mass spectrometry, various MALDI matrices have been identified and proven to be useful for achieving higher ion yield and/or mass resolution when different types of molecules are analyzed [7]. For instance, 2,5-dihydroxybenzoic acid (DHB) is the preferred MALDI matrix for measuring protein molecules. Whereas, 3-hydroxypicolinic acid (3-HPA) is the MALDI matrix for oligonucleotide measurements [17]. To ensure the approach of using MAC ions as internal references is applicable when other MALDI matrices are used, the driftscopes of several commonly used MALDI matrices were acquired. To further demonstrate the applicability of MAC ions in different polarity, the positively and negatively charged MAC ions were measured separately. All the results are shown in Fig. 2. In each case, a trend line is observed. Similar to the driftscope in Fig. 1b, with adequate zooming, unique vertical bands at specific m/z ratios can be detected which correspond to unique MAC ions generated from the particular MALDI matrix being measured. In comparison of the results obtained from using either positive or negative ion mode, lower signals were detected from the negatively charged

MAC ions. Since a different set of MAC ions were detected in the opposite polarity, the differences in signal intensity are expected according to our previous experience from using MALDI mass spectrometry.

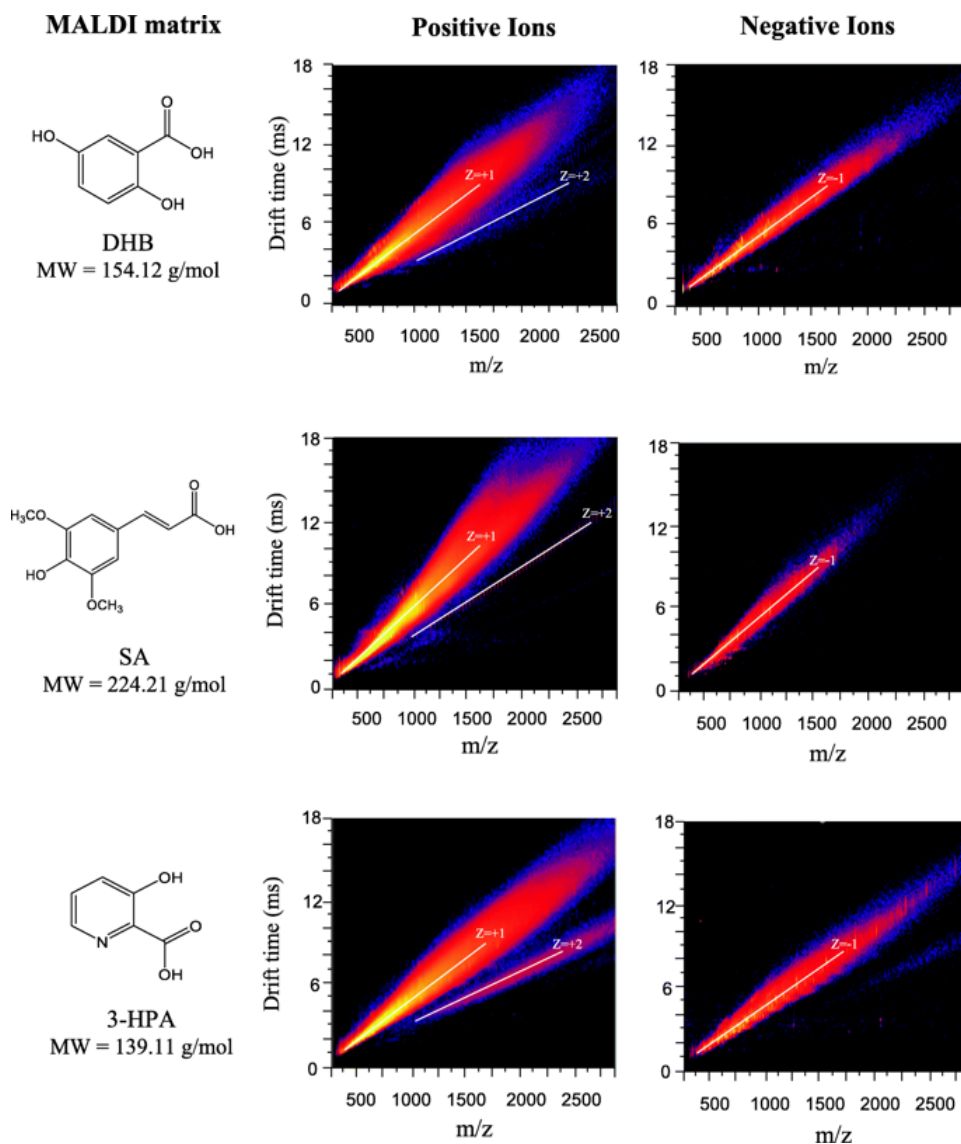


Figure 2. Molecular structure of selected MALDI matrices, and their corresponding driftscopes acquired in both positive and negative polarity. DHB = 2,5-dihydroxybenzoic acid; SA = sinapic acid; 3-HPA = 3-hydroxypicolinic acid. The solid white line(s) in each driftscope represents the trendline of corresponding matrix cluster ions with the same electrical charge (z)

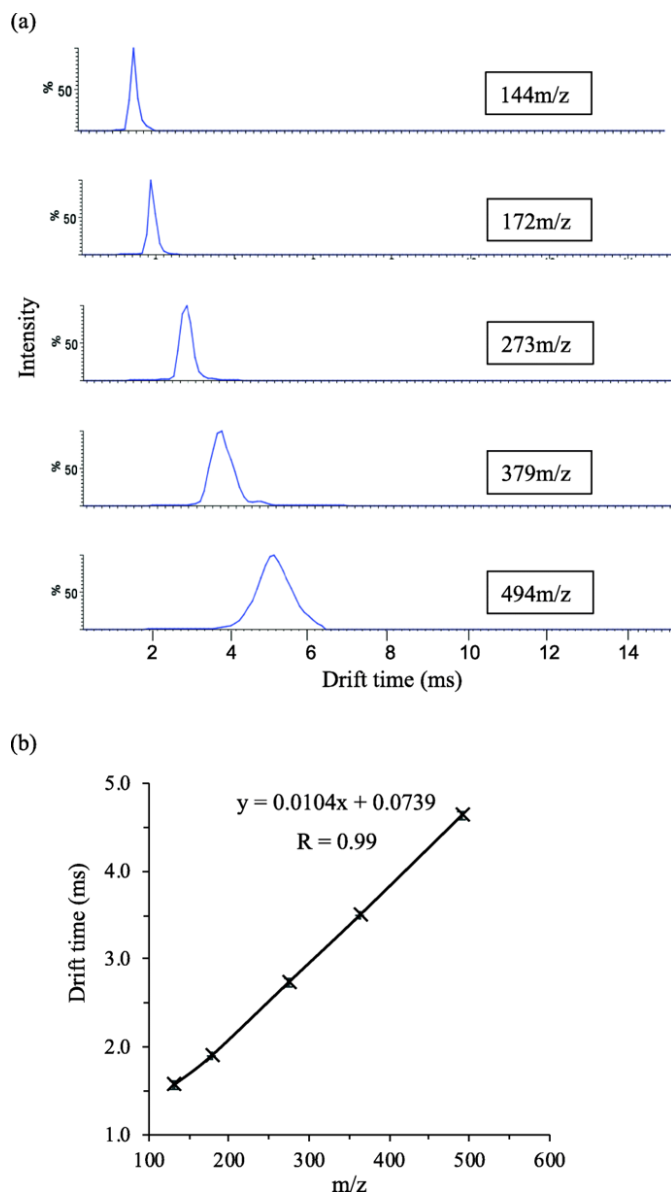


Figure 3. (a) Extracted ion mobilograms with specific mass-to-charge (m/z) ratios. All data were obtained from the driftscope of CHCA matrix in Fig. 1. (b) The drift time of each selected CHCA cluster ion is plotted as a function of their corresponding mass-to-charge ratio. The correlation function and coefficient (R) of the two experimental parameters are included

To demonstrate MAC ions can be used as internal references for ion mobility mass spectrometric measurements, the relationship between the m/z ratio of MAC ions and their corresponding drift time was evaluated. A series of discrete bands with relative high signal intensity in the driftscope of CHCA matrix (Fig. 1b) was selected. Each band represents a unique MAC ion of CHCA matrix. The accurate m/z ratio of each selected MAC ion could be easily looked up from the annotated mass spectrum of CHCA matrix after it had been aligned with the CHCA driftscope. To determine the accurate drift time of each selected MAC ion, the m/z ratio of each selected MAC ion was used to extract an ion mobilogram from the CHCA driftscope. The results are shown in Fig. 3a. With the increase on the m/z ratio of selected MAC ions, the corresponding

drift time are increased. By plotting the data in Fig. 3b, a clear linear relationship between the m/z ratio of selected MAC ions and their corresponding drift time can be identified, which complies with the principle of traveling wave ion mobility being used in this study. To further characterize the relationship between the m/z ratio of MAC ions and their corresponding drift time, the experimental results in Fig. 3b were compared with another set of experimental results obtained from a series of polymer standards with increasing CCS while their m/z ratios are similar to the MAC ions. By overlaying the results of CHCA matrix with those obtained from the polymer standards, which were measured in separate experiments, it shows the two sets of results correlate well with each other (Fig. 4). Hence, MAC ions do possess compatible mobility characteristics, thus meeting the basic requirement to be used as internal references.

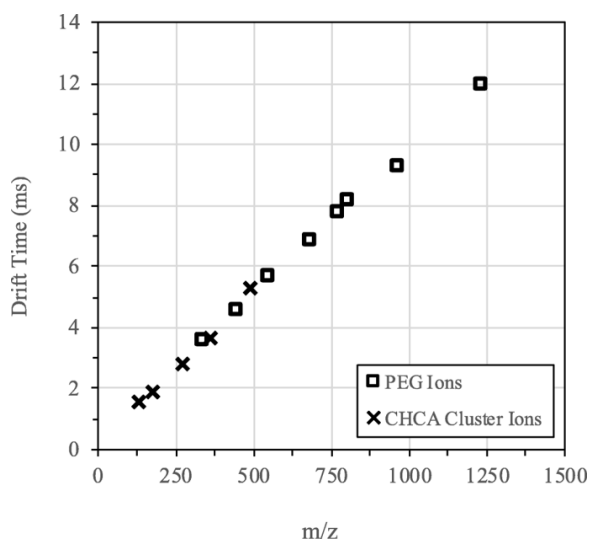


Figure 4. Comparison of the correlation between mass-to-charge ratio and drift time of CHCA cluster ions to that obtained from the polyethylene glycol (PEG) standards

Applications of MAC internal references

Similar to using the signal acquired from an external calibrant or standard to confirm the proper operation on sample ionization, ion transmission and ion detection when setting up a new ion mobility experiment, the detection of MAC ions can provide the same information. This is due to the fact that the MALDI matrix is mixed with each sample during the sample preparation process, thus MAC ions are generated simultaneously when the sample is ionized. An example from measuring a small peptide sample, in which CHCA matrix was used, is shown in Fig. 5a. Besides confirming the actual performance of ion mobility measurements had reached or exceeded the expected level, the presence of MAC ions in the driftscope could also provide an assistance for trouble shooting false negative results. To demonstrate the presence of MAC ions do not interfere or suppress the signal of target ions, the concentration of peptide sample being used in this study was intentionally kept at a low level, approximately a thousand times lower concentration than that of MALDI matrix. The band that corresponds to the peptide ion can still be easily identified in the driftscope (Fig. 5a). For future applications of MAC ions, it is possible that the ion of interest from other samples may have very similar m/z ratios or drift time as the MAC ions. If so, this will result in the overlapping of signals in the driftscope. Firstly, the overlapped signals should have higher intensity, thus the issue can be easily identified. Secondly,

a simple yet practical solution for overcoming this issue is to switch out the MALDI matrix with another one. As mentioned, different MALDI matrices generate different MAC ions, thus not overlapping with the signals of interest. Thirdly, for the longer term, with further improvement on the resolution of CCS, it will become possible to resolve the overlapped signals. As shown in the example of CHCA matrix, there are more than one specific MAC ions available for serving as internal references. Theoretically speaking, the minimum requirement for achieving the purpose of internal reference is the detection of only one specific MAC ion. Hence, we expect the approach of using MAC ions as internal references is applicable to a wide variety of ion mobility measurements.

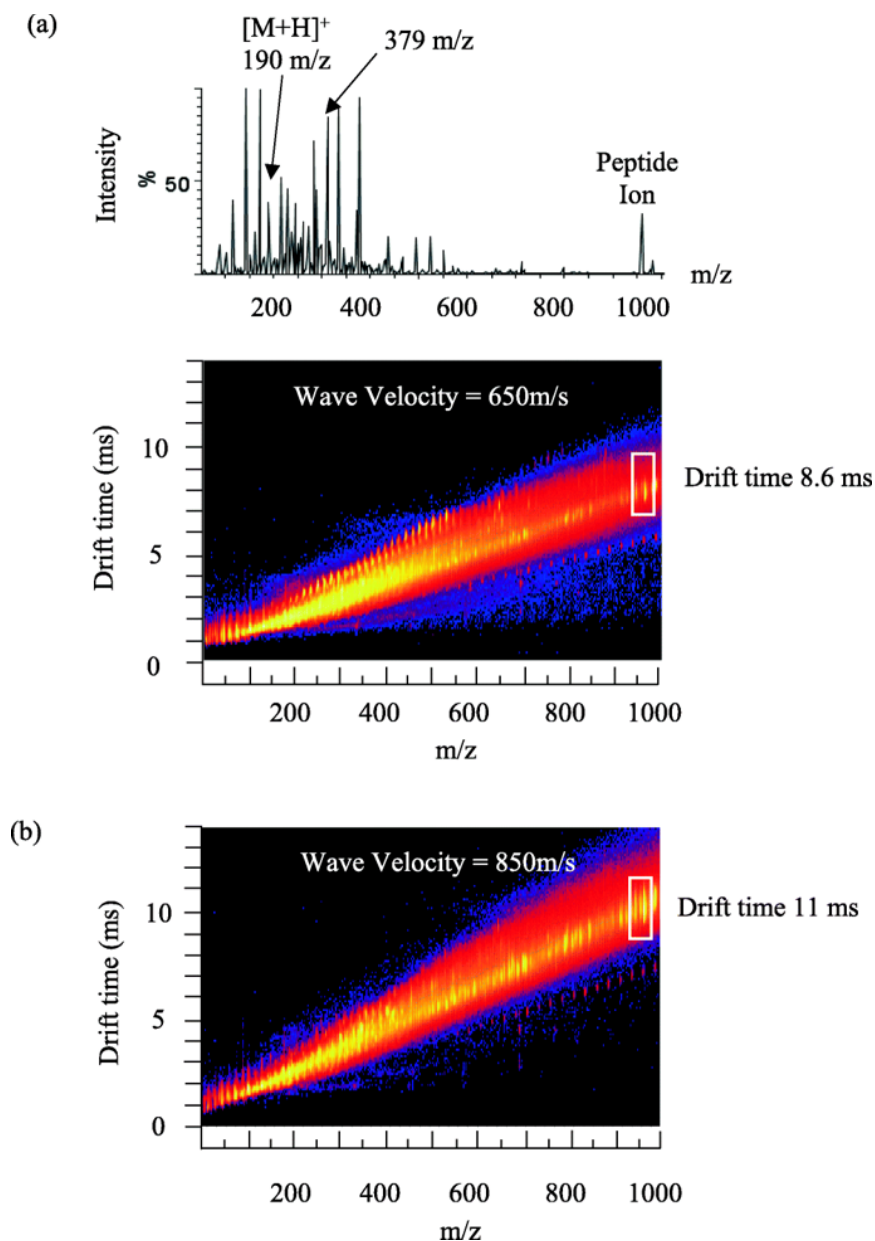


Figure 5. (a) MALDI mass spectrum and driftscope of selected peptide ion. CHCA matrix was used. (b) Driftscope of same peptide ion acquired at different wave velocities. The band that corresponds to the peptide ion is highlighted in the box

Complex analytical instrumentation including IM-MS are handmade. As a result, different units of the same model are not always made equally and their analytical performance may not be identical. This could be the reason for some of the failures on reproducing the same experimental results when a different unit without proper calibration was used. On the other hand, the analytical performance of the same unit could be shifted over time and/or usage, thus prohibiting the same experimental results to be acquired in repeated experiments unless preventive maintenance service is performed. Both are serious issues, but can be easily identified and compensated if internal references like MAC ions are used. For the purpose to demonstrate the potential benefit on using the MAC ions as internal references, the shifting on drift time measurement was simulated by manually adjusting the setting of wave velocity and the same peptide sample was re-measured. As shown in Fig. 5b, the drift time of the peptide ion was shifted from 8.6 ms to 11 ms. The drift time of MAC ions are also shifted in the same direction. For instance, the MAC ions with 379 m/z was shifted from 4.0 ms to 5.0 ms. The ratio of drift time between the ion of interest (i.e. peptide ion) and any selected MAC ion should theoretically stay constant when the performance is shifted. In the case of the results in Fig. 5b, the ratios of drift time are equal to each other ($8.6/4.0 = 11/5.0 = 2.2$). Mathematically, this shows how the detection of MAC ions can provide an assistance to determine whether two different drift time at 8.6 ms and 11 ms in Fig. 5a and b, respectively, correspond to the same peptide ion. If the signals that correspond to MAC ions were absent and the change in drift time did actually happen as a result of an unexpected variation of performance, it could lead to an incorrect data interpretation. In addition, we do expect this approach to interpret the experimental data will facilitate the transfer of specific experimental protocols between instruments and/or laboratories.

Conclusions

Instead of relying on calibration with suitable calibrants or developing a new strategy to achieve external calibration for ion mobility measurements, we report an alternative experimental approach to ensure proper ion mobility measurements can be carried out. In this approach, the intrinsic MAC ions that are generated during the MALDI process are used as internal references for ion mobility measurements. Among various MALDI matrices that have been commonly used in MALDI mass spectrometry, unique MAC ions with specific m/z ratios were detected in both polarities. There is a linear relationship between the measured drift time of MAC ions and their corresponding m/z ratios, which complies with the expected outcome of ion mobility spectrometry. Samples with relative low concentration and even the possible overlapping of signals with the MAC ions do not hinder the use of MAC ions as internal references. With the MAC ions, it facilitates the transfer of specific experimental protocols between instruments and/or laboratories and ensures the same experimental results are reproducible. Overall, we expect the approach of using MAC ions as internal references is applicable to a wide variety of ion mobility or related spectrometric measurements.

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References

1. Tyndall AM., Starr LH, Powell CF (1928) The mobility of ions in air. Part IV. Investigations by two new methods. *Proc. R. Soc. London, Ser. A*; 121:172–184
2. D'Atri V, Causon T, Hernandex-Alba O, Mutabazi A, Veuthey J, Cianferani S, Guillaume D (2018) Adding a new separation dimension to MS and LC-MS: what is the utility of ion mobility spectrometry? *J Sep Sci* 41(1):20–67
3. May JC, McLean JA (2015) Ion mobility-mass spectrometry: time-dispersive instrumentation. *Anal Chem* 87:1422–1436
4. Ewing MA, Glover MS, Clemmer DE (2016) Hybrid ion mobility and mass spectrometry as a separation tool. *J Chromatogr A* 1439:3–25
5. Cumeras R, Figueras E, Davis CE, Baumbach JI, Gràcia I (2015) Review on ion mobility spectrometry. Part 1: current instrumentation. *Analyst*. 140(5):1376–1390
6. Improved MALDI imaging quality and speed using the MALDI Synapt G2-Si HDMS. http://www.waters.com/waters/library.htm?cid=134740682&lid=134777497&locale=en_US. Accessed 5 Oct 2018
7. Hillenkamp F, Jaskolla TW, Karas M. (2013) The MALDI process and method. In: Hillenkamp, F, Peter-Katalinic J, editors. *MALDI MS: A practical guide to instrumentation, methods and applications*. 2nd Edition. Wiley-Blackwell. pp. 1–40
8. Knochenmuss R. (2010) MALDI ionization mechanisms: an overview. In: Cole RB. *Electrospray and MALDI Mass Spectrometry*. 2nd Edition. p. 149–83
9. Shahgholi M, Garcia BA, Chiu NH, Heaney PJ, Tang K (2001) Sugar additives for MALDI matrices improve signal allowing the smallest nucleotide change (a:T) in a DNA sequence to be resolved. *Nucleic Acids Res* 29:e91
10. Stow SM et al (2017) An interlaboratory evaluation of drift tube ion mobility – mass spectrometry collision cross section measurements. *Anal Chem* 89(17):9048–9055
11. Hines KM et al (2016) Evaluation of collision cross section calibrants for structural analysis of lipids by traveling wave ion mobility-mass spectrometry. *Anal Chem* 88:7329–7336
12. Lippens JL et al (2016) Modular calibrant sets for the structural analysis of nucleic acids by ion mobility mass spectrometry. *Analyst* 141:4084–4099

13. Maestre RF (2017) Calibration of the mobility scale in ion mobility spectrometry: the use of 2,4-lutidine as a chemical standard, the two-standard calibration method and the incorrect use of drift tube temperature for calibration. *Anal Methods* 9:4288–4292
14. Haler JRN et al (2017) Comprehensive ion mobility calibration: poly(ethylene oxide) polymer calibrants and general strategies. *Anal Chem* 89:12076–12086
15. Duez Q et al (2017) Polymers for traveling wave ion mobility spectrometry calibration. *J Am Soc Mass Spectrom* 28:2483–2491
16. Krause J, Stoeckli M, Schlunegger UP (1996) Studies on the selection of new matrices for ultraviolet matrix-assisted laser desorption/ ionization time-of-flight mass spectrometry. *Rapid Commun Mass Spectrom* 10(15):1927–1933
17. Wambua D, Ubukata M, Dane J, Cody RB (2014) Chiu NH*. Bottom-up mass spectrometric sequencing of microRNA. *Anal. Methods*. 6:8829–8839